REVIEW ARTICLE

Diet-induced obesity: rodent model for the study of obesity-related disorders

Tiago Campos Rosini[1], Adelino Sanchez Ramos da Silva[2], Camila de Moraes[3]

[1] Student of Physical Education and Sports, Escola de Educação Física e Esporte de Ribeirão Preto, Universidade de São Paulo (EEFERP-USP), São Paulo, SP, Brazil
[2] Post-doctorate, Laboratory of Clinical Investigation in Insulin Resistence; Professor Doctor, EEFERP-USP, SP, Brazil
[3] PhD in Motility Sciences; Professor Doctor, EEFERP-USP, Ribeirão Preto, SP, Brazil

Summary

Obesity has been significantly increasing worldwide, and environmental factors such as excessive food intake and sedentary lifestyle are the main factors related to the genesis of this disease. In laboratory animals, the genesis of obesity is related mostly to genetic mutations, but this model is far from that found in humans. The use of hypercaloric or hyperlipidemic diets has been used as a model of obesity induction in animals, because of its similarity to the genesis and metabolic responses caused by obesity in humans. The objective of this review is to show the different types of diets used to induce obesity in rodents, the induced metabolic alterations, and to identify some points that should be taken into account so that the model can be effective for the study of obesity-related complications. A search was performed in the PubMed database using the following keywords: 1- "hypercaloric diet" AND "rodent", 2- "hyperlipidic diet" AND "rodent", selecting those considered the most relevant according to the following criteria: date of publication (1995-2011); the use of wild-type animals; detailed description of the diet used and analysis of biochemical and vascular parameters of interest. References were included to introduce subjects such as the increased prevalence of obesity and questions related to the genesis of obesity in humans. The model of diet-induced obesity in rodents can be considered effective when the objective is the study of the physiopathology of metabolic and vascular complications associated with obesity.

Keywords: Obesity; cardiovascular diseases; lipid metabolism disorders.

Study conducted by the Study and Research Group in Exercise and Metabolism Physiology of the Escola de Educação Física e Esporte de Ribeirão Preto, Ribeirão Preto, SP, Brazil

Submitted on: 07/06/2011
Approved on: 02/10/2012

Financial Support:
Pró-reitoria de Pesquisa da Universidade de São Paulo, SP, Brazil

Correspondence to:
Camila de Moraes
Avenida Bandeirantes, 3900
Monte Alegre
Ribeirão Preto – SP, Brazil
CEP: 14040-907
camimoraes@usp.br

Conflict of interest: None.

Introduction

The incidence of cardiovascular and metabolic diseases in the world population is growing, and their higher prevalence in obese individuals has attracted the attention of health professionals and researchers. Many population-based studies have shown that excess adipose tissue, mainly in the abdomen, is closely related to risk of cardiovascular complications, such as the development of coronary artery disease and hypertension. Furthermore, excess fat also causes some metabolic abnormalities such as dyslipidemias, insulin resistance, and diabetes mellitus type II. Environmental factors, including inadequate diets and a sedentary lifestyle, are the major factors that contribute to the genesis of obesity in humans.

The study of the mechanisms by which obesity induces physiological dysfunctions can be facilitated by using an animal model in the research environment. There are various types of animal models, usually rodents, who develop obesity due to genetic mutations. However, considering that the model should be as close as possible to the genesis of obesity in humans, induction of this condition via consumption of highly palatable, high-calorie foods may be more appropriate.

Therefore, the objective of this study is to show different obesity-induction protocols in rodents via consumption of palatable diets, and compare the metabolic and vascular dysfunctions induced by these diets.

Methods

A search was performed in the PubMed database using the following key words: 1-"hypercaloric diet" AND "rodent", 2- "hyperlipidic diet "AND "rodent".

A total of 100 publications were retrieved through this search, and those considered most relevant by the authors of this review were selected, using the following criteria: publication date between 1995 and 2011, use of wild-type animals, detailed description of the diet used in the study and analysis of biochemical and vascular parameters of interest. Additionally, references of population studies were included to introduce subjects such as the increased prevalence of obesity and questions related to the genesis of obesity in humans.

Factors related to the genesis of obesity

The increased prevalence of cases of overweight and obesity worldwide has occurred proportionally to the progressive decrease of the energy spent on work activities, in the performance of household chores, and on the daily needs. Moreover, the supply of highly palatable foods has contributed to an increase in the obese population[1].

The causes of obesity in the population are multiple and complex. To some authors, the influence of the environment is the primary cause, as the human genotype has not changed substantially over the past three decades. Thus, small changes in daily life, such as the use of machines for washing clothes and dishes, and the use of cars for transportation can have a significant impact on the total daily energy spent. In addition to this reduction in the total energy use due to low physical activity, it appears that environmental factors stimulate greater energy intake, through excess fat in diet, consumption of high-calorie food, large-sized portions, frequency of food intake, and lower cost and greater availability of food[2].

The relationship between obesity and chronic stress has been studied. The exposure of mice to a model of social stress increased circulating levels of ghrelin (the peptide responsible for the sensation of hunger) in these animals, by mechanisms not yet understood. Ghrelin interacts with its receptor (GHSR) located in the catecholaminergic brain neurons, leading to a decrease in the state of depression observed in animals exposed to social stress. At the same time, these animals showed a picture of hyperphagia and increase in body weight[3].

Other studies have supported the hypothesis that obesity is determined by genetic factors in 50% to 90% of cases, and that the environment only determines the phenotypic expression[4]. The consensus is that the genetic factor alone is not the cause of obesity. Cases of genetic mutations (such as the deletion of genes that regulate the production of leptin, the satiety hormone) are rare. However, cases of polymorphisms that alter the production of hormones regulating food intake and energy expenditure are being detected in the population, and the polymorphism associated with environmental factors such as sedentary lifestyle[5] and excess carbohydrate[6] and saturated fat intake[7] increases the risk for the development of obesity.

Unlike humans, the genesis of obesity in laboratory animals is mostly related to genetic modifications that can alter or suppress the secretion of neuropeptides, hormones related to satiety, or metabolism. Furthermore, according to the modified gene, the animals will develop obesity early or late, together with other disorders such as insulin resistance, diabetes, hypercholesterolemia, hypertension, and male infertility, which allows the investigation of the physiopathology of obesity and its comorbidities. Currently, animal models have been used to investigate candidate genes and to confirm the cause of obesity and other diseases. This is based on the investigation of the genetic sequence of individuals who have a certain disease when compared to their healthy peers.

The determination of the candidate gene and the study of gene function in mice provide, in addition to a possible confirmation of gene function, the possible development of genetically-engineered animals that will have problems and characteristics similar to those of humans suffering from certain diseases[8]. Further studies and the

dissemination of technology will allow this methodology to be available to researchers in a few years.

To date, animal models of obesity based on gene modification are very distinct from the genesis of obesity in humans, as there are only rare cases of obese individuals with a genetic mutation. The secretion of leptin, a hormone secreted by adipocytes, is a good example of the difference between the genesis of obesity in animals and humans. Leptin is related to the appetite reduction that occurs through the inhibition of appetite-related neuropeptide formation, such as neuropeptide Y, and also through the increase of the expression of anorectic neuropeptides, such as α- melanocyte stimulating hormone (α-MSH), corticotropin-releasing hormone (CRH), and substances synthesized in response to amphetamine and cocaine at the level of the central nervous system[9]. In laboratory animals, such as ob/ob mice (with mutation in the ob gene), leptin levels are greatly reduced, which results in hyperphagia and consequent obesity. When these animals are treated with leptin, food intake is decreased, resulting in weight loss[10]. However, in obese individuals, plasma levels of leptin are greatly elevated, approximately five times higher than those found in lean subjects, suggesting a possible central resistance to this hormone[11].

Moreover, the administration of leptin in humans has not proved to be effective in reducing obesity. These contrasts between data obtained in laboratory animals and humans indicate that the mechanisms that control metabolism and body weight are more complex than what was expected, and that further investigations related to the genus and species are needed[12].

Diet-induced obesity and metabolic and vascular alterations

Adopting a hypercaloric or hyperlipidemic diet has been widely used as a template to induce obesity in lab animals. This particular model is extremely useful in research on obesity in laboratory animals due to its close resemblance to the genesis and metabolic responses caused by obesity in humans, i.e., obesity is the consequence of a positive energy balance generated by environmental factors, such as, for instance the excessive intake of high-calorie foods and a sedentary lifestyle[13].

Hyperlipidemic diets are known to be directly related to the development of obesity[14]. Recently, it was demonstrated that long-chain saturated fatty acids, mainly found in red meat, are the most harmful lipids regarding accumulation of adipose mass[15]. In this study, the researchers found that these molecules bind to Toll-like receptors (TLR2 and TLR4) of microglia, cells that protect the hypothalamus, stimulating the production of pro-inflammatory cytokines (TNF-alpha, IL-1β, and IL-6) and, consequently, causing the destruction of neurons responsible for appetite control and thermogenesis.

Some studies have demonstrated the effectiveness of a hypercaloric diet or hyperlipidemic diet in the genesis of obesity and its comorbidities, particularly in pigs[16] and Sprague-Dawley rodents[17,18]. Wistar rats are also used in studies where obesity is diet-induced, and the results have shown that the body weight is increased. Wistar rats treated with a hypercaloric and hyperlipidemic diet for three months had an approximate 1.4-fold increase in body weight when compared with control animals[19].

On the other hand, results on insulin alterations are conflicting. Some investigations have found that the diet can increase insulin levels[20-23,] whereas others showed no difference[24,25]. Regarding glycemia, few studies have reported a significant increase of this biochemical parameter[21,26].

The model of diet-induced obesity in Wistar rats has also been used to investigate endothelial dysfunctions, as most of the studies of animals submitted to this treatment demonstrate important metabolic abnormalities such as increased triglycerides, which are related to an increased production of superoxide anions and subsequent reduction in the bioavailability of nitric oxide, an important vasodilator released by the vascular endothelium.

In the obesity model, some studies have shown that consumption of diets rich in fat and sucrose for three days and 12 weeks leads to a decreased time-dependent vasodilator response to endothelium-mediated action (carbachol) or direct action on smooth vascular muscle (sodium nitroprusside) in the mesenteric artery of Wistar rats, showing that reductions in endothelial function may be more related to increased levels of triglycerides[27,28]. The mechanisms through which obesity promotes reduction of the relaxation response are yet to be clarified. Some authors suggest a direct association between endothelial dysfunction and dyslipidemia, that is, high levels of triglycerides and cholesterol fractions (mainly LDL-c) would cause damage to the endothelial cell with lower nitric oxide production, resulting in arterial hypertension and thromboembolic disorders[23,29].

Guidelines to increase the effectiveness of the diet-induced obesity model

For the model of diet-induced obesity to be effective, some measures related to the environment where the animal is kept must be observed, especially when rodents without genetic mutations are studied, such as Wistar rats. The number of animals per box should not be greater than four, and if the vivarium structure allows it, small increases in ambient temperature and length of darkness in the light-dark cycle may facilitate the pathogenesis of obesity. The increase in temperature would reduce the body energy expenditure that the animal would have to spend to maintain body temperature in the case of cooler environments and thus, a positive energy balance would be generated.

Moreover, rodents are nocturnal animals and thus, an increase in the darkness period of the vivarium cycle would provide more time for food intake, especially if the diet is highly palatable and is presented in containers on the box floor[13].

The animal's age at the beginning of the experimental protocol can interfere with body mass gain. Young animals have different metabolism that results in greater gain in lean mass, so it is advisable that older animals, aged approximately 100 days, are submitted to diet-induced obesity[13]. However, there have been studies in which young animals fed a high-calorie diet for a long period of time increased body weight in comparison with the control group[21]. It seems that the type of diet is another intervening factor for weight gain in animals of different ages. Animals with similar weight and age at the start of the experimental protocol showed different results regarding body weight gain. Some studies have demonstrated

Table 1 – Different types of diet used and diet-induced body and metabolic alterations

Reference	Age/initial weight/ gender	Type of diet	Time of ingestion	Weight	GLU	INS	LEP	TG
Barnes et al. (2003)[26]	250 - 275 g M	Hyperlipidic diet (soybean oil added)	12 weeks	+	+	+	+	0
Burneiko et al. (2006)[31]	180 - 200 g M	Hypercaloric diet (peanuts, milk chocolate, and corn biscuits)	8 weeks	-	0	0	0	+
DeSchepper et al. (1998)[20]	200 g M	Cafeteria diet (biscuits, salami, butter, cheese, and bacon)	20 weeks	+	0	+	+	0
Estadela et al. (2004)[25]	81 days M	Hyperlipidic diet (peanuts, milk chocolate, and sugar cookies)	8 weeks	+	-	-	+	+
Guerra et al. (2007)[30]	210-230 g M	Cholesterol-rich diet (1% cholesterol + 0.25% cholic acid added)	8 weeks	+	0	0	0	+
Lopez et al. (2003)[24]	40 days M	Cafeteria diet (pate, French fries, chocolate, bacon, and biscuits)	8 weeks	+	-	-	+	-
Moraes et al. (2007)[22]	270 – 300 g M	Hypercaloric diet (AIN93 plus sweetened condensed milk and sucrose)	8 weeks	+	-	+	0	+
Moraes et al. (2008)[23]	295 – 310 g M	Hypercaloric diet (AIN93 plus sweetened condensed milk and sucrose)	8 weeks	+	+	+	0	+
Naderalli et al. (2001)[27]	200 - 210 g F	Hypercaloric diet (sweetened condensed milk and sucrose)	12 weeks	+	-	-	+	+
Naderalli et al. (2003)[28]	180-200 g M	Hypercaloric diet (sweetened condensed milk and sucrose)	15 weeks	+	-	+	+	+
Nascimento et al. (2008)[21]	105-120 g M	Hypercaloric diet (peanuts, casein, soybean oil, chocolate + corn biscuits or chips or instant noodles + grated cheese or sweetened condensed milk + wafer biscuits)	14 weeks	+	+	+	+	+
Silva et al. (2010)[19]	30 days M	Hyperlipidic diet (corn starch, casein, sucrose, dextrin powder, lard, soybean oil, cellulose, minerals and vitamins mix, cystine, and choline)	12 weeks	+	-	+	0	0
Zambon et al. (2009)[32]	225 g M	Hypercaloric diet (peanuts, milk chocolate, and corn starch biscuits)	3 weeks	-	0	0	0	+

M, male; F, female; GLU, glucose; INS, insulin; LEP, leptin; TG, triglycerides; +, increase in relation to controls; -, no difference in relation to controls; 0, not quantified.

an increase in body weight[24,30], while others have not[31,32], demonstrating that the type of diet can influence the genesis of obesity (Table 1).

The use of the model of diet-induced obesity in animals has shown to be effective for the study of the physiopathology of complications associated with obesity, such as cardiovascular disease and more specifically those of the endothelial function, as it is the closest template to the genesis of obesity in humans.

Acknowledgements

The authors acknowledge the Universidade de São Paulo for the Scientific Initiation Grant given to Tiago C. Rosini.

References

1. Bouchard C. A epidemia da obesidade: Introdução. In: Bouchard C. Atividade física e obesidade. Translation: Dulce Marino. São Paulo: Manole, 2003. p. 3-22.
2. Hill JO, Melanson EL. Overview of the determinants of overweight and obesity: current evidence and research issues. Med Sci Sports Exerc. 1999;31(Suppl):S515-21.
3. Chuang JC, Perello M, Sakata I, Osborne-Lawrence S, Savitt JM, Lutter M, et al. Ghrelin mediates stress-induced food-reward behavior in mice. J Clin Invest. 2011;121(7):2684-92.
4. Barsh GS, Farooqi IS, O´Rahilly S. Genetics of body-weight regulation. Nature. 2000;6;404(6778):644-51.
5. Ochoa MC, Moreno-Aliaga MJ, Martínez-González MA, Martínez JA, Marti A. TV watching modifies obesity risk linked to the 27Glu polymorphism of the ADRB2 gene in girls. Int J Pediatr Obes. 2006;1(2):83-8.
6. Martínez JA, Corbalán MS, Sánchez-Villegas A, Forga L, Marti A, Martínez-González MA. Obesity risk is associated with carbohydrate intake in women carrying the Gln27Glu beta2-adrenoceptor polymorphism. J Nutr. 2003;133(8):2549-54.
7. Memisoglu A, Hu FB, Hankinson SE, Manson JE, De Vivo I, Willett WC, et al. Interaction between a peroxisome proliferator-activated receptor gamma gene polymorphism and dietary fat intake in relation to body mass. Hum Mol Genet. 2003;12(22):2923-9.
8. Cox RD, Church CD. Mouse models and the interpretation of human GWAS in type 2 diabetes and obesity. Dis Model Mech. 2011;4(2):155-64.
9. Friedmann JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998;395(6704):763-70.
10. Wilding JP. Leptin and the control of obesity. Curr Opin Pharmacol. 2001;1(6):656-61.
11. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334(5):292-5.
12. Lee DW, Leinung MC, Rozhavskaya-Arena M, Grasso P. Leptin and the treatment of obesity: its current status. Eur J Pharmacol. 2002;440(2-3):129-39.
13. Tschöp M, Heiman ML. Rodent obesity models: an overview. Exp Clin Endocrinol Diabetes. 2001;109(6):307-19.
14. Velloso LA. The brain is the conductor: diet-induced inflammation overlapping physiological control of body mass and metabolism. Arq Bras Endocrinol Metabol. 2009;53(2):151-8.
15. Milanski M, Degasperi G, Coope A, Morari J, Denis R, Cintra DE, et al. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J Neurosci. 2009;29(2):359-70.
16. Thomas TR, Pellechia J, Rector RS, Sun GY, Sturek MS, Laughlin MH. Exercise training does not reduce hyperlipidemia in pigs fed a high-fat diet. Metabolism. 2002;51(12):1587-95.
17. Petry CJ, Ozanne SE, Wang CL, Hales CN. Effects of early protein restriction and adult obesity on rat pancreatic hormone content and glucose tolerance. Horm Metab Res. 2000;32(6):233-9.
18. Dobrian AD, Davies MJ, Prewitt RL, Lauterio TJ. Development of hypertension in a rat model of diet-induced obesity. Hypertension. 2000;35(4):1009-15.
19. Da Silva AS, Pauli JR, Ropelle ER, Oliveira AG, Cintra DE, De Souza CT, et al. Exercise intensity, inflammatory signaling and insulin resistance in obese rats. Med Sci Sports Exerc. 2010;42(12):2180-8.
20. De Schepper JA, Smitz JP, Zhou XL, Louis O, Velkeniers BE, Vanhaelst L. Cafeteria diet-induced obesity is associated with a low spontaneous growth hormone secretion and normal plasma insulin-like growth factor-I concentrations. Growth Horm IGF Res. 1998;8(5):397-401.
21. Nascimento AF, Sugizaki MM, Leopoldo AS, Lima-Leopoldo AP, Luvizotto RA, Nogueira CR, et al. A hypercaloric pellet-diet cycle induces obesity and comorbidities in wistar rats. Arq Bras Endocrinol Metabol. 2008;52(6):968-74.
22. De Moraes C, Camargo EA, Antunes E, de Nucci G, Zanesco A. Reactivity of mesenteric and aortic rings from trained rats fed with high caloric diet. Comp Biochem Physiol A Mol Integr Physiol. 2007;147(3):788-92.
23. De Moraes C, Davel AP, Rossoni LV, Antunes E, Zanesco A. Exercise training improves relaxation response and SOD-1 expression in aortic and mesenteric rings from high caloric diet-fed rats. BMC Physiol. 2008;29:8-12.
24. López IP, Marti A, Milagro FI, Zulet Md Mde L, Moreno-Aliaga MJ, Martinez JA, et al. DNA microarray analysis of genes differentially expressed in diet-induced (cafeteria) obese rats. Obes Res. 2003;11(2):188-94.
25. Estadella D, Oyama LM, Dâmaso AR, Ribeiro EB, Oller do Nascimento CM. Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats. Nutrition. 2004;20(2):218-24.
26. Barnes MJ, Lapanowski K, Conley A, Rafols JA, Jen KL, Dunbar JC. High fat feeding is associated with increased blood pressure, sympathetic nerve activity and hypothalamic mu opioid receptors. Brain Res Bull. 2003;61(5):511-9.
27. Naderali EK, Brown MJ, Pickavance LC, Wilding JP, Doyle PJ, Williams G. Dietary obesity in the rat induces endothelial dysfunction without causing insulin resistance: a possible role for triacylglycerols. Clin Sci (Lond). 2001;101(5):499-506.
28. Naderali EK, Williams G. Prolonged endothelial-dependent and -independent arterial dysfunction induced in the rat by short-term feeding with a high-fat, high-sucrose diet. Atherosclerosis. 2003;166(2):253-9.
29. Kusterer K, Pohl T, Fortmeyer HP, März W, Scharnagl H, Oldenburg A, et al. Chronic selective hypertriglyceridemia impairs endothelium-dependent vasodilatation in rats. Cardiovasc Res. 1999;42(3):783-93.
30. Guerra RL, Prado WL, Cheik NC, Viana FP, Botero JP, Vendramini RC, et al. Effects of 2 or 5 consecutive exercise days on adipocyte area and lipid parameters in Wistar rats. Lipids Health Dis. 2007;2:6-16.
31. Burneiko RC, Diniz YS, Galhardi CM, Rodrigues HG, Ebaid GM, Faine LA, et al. Interaction of hypercaloric diet and physical exercise on lipid profile, oxidative stress and antioxidant defenses. Food Chem Toxicol. 2006;44(7):1167-72.
32. Zambon L, Duarte FO, Freitas LF, Scarmagnani FR, Dâmaso AR, Oliveira-Duarte AC, et al. Efeitos de dois tipos de treinamento de natação sobre a adiposidade e o perfil lipídico de ratos obesos exógenos. Rev Nutr. 2009;22(5):707-15.